1973

Avoidance learning in Drosophila melanogaster

William Lucas

University of the Pacific

Follow this and additional works at: https://scholarlycommons.pacific.edu/uop_etds

Part of the Life Sciences Commons

Recommended Citation
https://scholarlycommons.pacific.edu/uop_etds/1831

This Thesis is brought to you for free and open access by the Graduate School at Scholarly Commons. It has been accepted for inclusion in University of the Pacific Theses and Dissertations by an authorized administrator of Scholarly Commons. For more information, please contact mgibney@pacific.edu.
AVOIDANCE LEARNING IN
DROSOPHILA MELANOGASTER

A Thesis
Presented to
The Faculty of the Graduate School
University of the Pacific

In Partial Fulfillment
of the Requirements for the Degree
Master of Arts

by
William Lucas
September 1973
This thesis, written and submitted by

William H. Lucas III

is approved for recommendation to the Committee on Graduate Studies, University of the Pacific.

Department Chairman or Dean:

[Signature]

Thesis Committee:

[Signature] Chairman

[Signature] Chairman

[Signature]

Dated 9/12/73
# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>LIST OF FIGURES</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chapter</td>
<td></td>
</tr>
<tr>
<td>I. INTRODUCTION</td>
<td>1</td>
</tr>
<tr>
<td>II. METHOD</td>
<td>10</td>
</tr>
<tr>
<td>Experiment I</td>
<td>10</td>
</tr>
<tr>
<td>Subjects</td>
<td>10</td>
</tr>
<tr>
<td>Apparatus</td>
<td>10</td>
</tr>
<tr>
<td>Procedure</td>
<td>11</td>
</tr>
<tr>
<td>Method of Data Analysis</td>
<td>12</td>
</tr>
<tr>
<td>Experiment II</td>
<td>13</td>
</tr>
<tr>
<td>Apparatus</td>
<td>13</td>
</tr>
<tr>
<td>Procedure</td>
<td>13</td>
</tr>
<tr>
<td>III. RESULTS</td>
<td>15</td>
</tr>
<tr>
<td>Experiment I</td>
<td>15</td>
</tr>
<tr>
<td>Experiment II</td>
<td>18</td>
</tr>
<tr>
<td>IV. DISCUSSION</td>
<td>20</td>
</tr>
<tr>
<td>V. SUMMARY</td>
<td>24</td>
</tr>
<tr>
<td>REFERENCES</td>
<td>25</td>
</tr>
</tbody>
</table>

ii
# LIST OF FIGURES

<table>
<thead>
<tr>
<th>Figure</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Performance of control vs experimental group over time, Experiment I</td>
<td>17</td>
</tr>
<tr>
<td>2.</td>
<td>Performance of control vs experimental group over time, Experiment II</td>
<td>19</td>
</tr>
</tbody>
</table>
I. INTRODUCTION

The fruit fly, Drosophila melanogaster has had a great impact on the field of genetic research due to its characteristics of being highly variable, prolific, easily maintained, and having a small number of large-sized chromosomes (Fuller & Thompson, 1960). Unfortunately, the same can not be said for its impact on behavioral research. Among the behaviors studied are phototaxis (Hirsch & Boudreau, 1958), geotaxis (Hirsch & Erlenmeyer-Kimling, 1962), mating behavior (Bastock, 1956), and habitat preference (Waddington, Woolf, and Perry, 1954). Murphey (1967) has observed that noticeably absent are any examples of successful instrumental conditioning, which are necessary for the fruit fly to become an important part of comparative psychology.

The importance of showing that Drosophila are capable of learning an instrumental response would, with the information already known from genetic studies, enable researchers to possibly isolate the genetic basis of a simple learning process and thus gain greater insight into this phenomenon. If a reliable technique can be found for learning in Drosophila, a new field of research would be opened which could greatly add to our knowledge of the evolution of behavior.
**Drosophila melanogaster** are members of the phylum arthropoda and class insecta. Adult insects differ from other arthropods in having usually two pairs of wings, *(Drosophila* has one pair and two vestigial knobs), three major body divisions, and never more than three pairs of legs, *(Drosophila* has three). (Murphey, 1967).

The central nervous system of *Drosophila* consists of a supracesophageal nerve mass, or brain connected with two ganglia, one located in the head and the other in the thorax. These are connected by the cervical connective which constitutes a ventral nerve cord (Murphey, 1967). The brain is composed of a protocerebrum which receives the antennal nerves and a tritocerebrum which gives rise to nerves that innervate the labrum (lower lip) and the digestive tract (Barnes, 1968).

Thorpe (1939) reported that when *Drosophila melanogaster* larvae were raised on a peppermint flavoured medium, which is usually aversive, the imagos (adults) exhibited a decreased aversion for the peppermint in comparison with the imagos of larvae raised without peppermint on a standard laboratory medium. At first Thorpe interpreted this phenomenon as a learning process, but later changed his hypothesis to consider it a habituation process. He hypothesized that the peppermint contained both menthol, which is at first repellant, and esters, which serve to attract the flies. The menthol might at first mask the presence of the esters, but as the flies habituate to the menthol the effect
of the esters would come into play, and the apparent appearance of a complete reversal from repulsion to attraction would occur (Thorpe, 1963).

Several tests were run by Hershberger and Smith (1967) to show that this decreased aversion to peppermint could be interpreted as true conditioning rather than habituation. They felt that by associating the scent of peppermint with a reinforcing stimulus such as food, and then taking away that reward while the flies were still in the presence of the peppermint, that they could test these same flies to see if this procedure decreased in these flies the effect of the peppermint over the control subjects who were only reinforced by the peppermint. If this occurred, then a conditioning interpretation could be valid.

They raised larvae in scented or unscented conditions and the imagos were then raised in either scented or non-scented environments reinforced by food. This was done by adding .5% of peppermint scent by volume to the regular medium in which the flies were raised. They also kept imagos for 24 hours in scented or unscented jars without medium. They then gave the flies a one-trial test in a Y-maze olfactometer to see to which arm, scented or unscented, they were attracted. They reported results similar to Thorpe's and interpreted the results as a form of learning, but Yeatman and Hirsch (1971) pointed out methodological discrepancies which put these results in doubt.
Using the same apparatus and a similar procedure, Manning (1967) ran a second trial on the flies which chose the scented arm on the first trial. He found that 46.7% of the peppermint scent reared flies chose the scented arm on the first trial, but then only 50.2% of these flies chose that arm again on the second trial. Eight generations were used with unchanged results. Manning also found that rewarding flies for choosing the peppermint scent had no effect on their subsequent choice, and after three trials the flies were going to the unscented arm of the maze in significant numbers. It appears that with these results, a habituation hypothesis is valid in olfactory conditioning.

The first report of instrumental conditioning in *Drosophila* was reported by Murphey (1967b). Using a T-maze and flies bred for negative geotaxis (a tendency to move away from gravity), and using the opportunity to move away from gravity as a reward, he found in his first experiment that flies could be trained to turn left or right. In a second experiment an effort was made to find a means of improving performance of the subjects over that of the first experiment. Three groups were used; group P was shocked for incorrect responses, group C had no shock but a correction of errors procedure was used. This procedure consisted of forcing the flies to choose the correct arm of the maze by blocking off the end of the incorrect arm so that they were forced to choose the correct arm to complete each trial. Group N consisted of scores from subjects in the first
experiments that reached criterion. These scores were then used as a comparison for the first two groups (P and C) to see if performance could be enhanced. Although punishment enhanced performance, in comparison to group N, the difference between groups P and C was not statistically significant.

In a third experiment, Murphey investigated delay of reward and magnitude of reward on performance. Two apparatuses were used, with varying lengths of T-maze arms to provide varying access to the opportunity to move away from gravity. Apparatus number one was the same as that used in the first experiment, with 15 mm arms. Apparatus number two was identical but had 30 mm arms. Magnitude of reward was manipulated by varying the length of the end chambers up which the subjects flew as its reward. Large magnitude of reward was defined as 100 mm, small magnitude of reward was defined as 35 mm in length. No difference was found in any of the four groups tested, although all groups learned. Unfortunately, as before, in Drosophila research, attempted replication of the study (Yeatman & Hirsch, 1971) met with failure. Yeatman & Hirsch (1971) also tried to breed a line of good and bad learners but obtained no significant results.

In trying to provide a more efficient way to train flies, Murphey (1969) studied the effects of 1) shock level for incorrect responses, 2) whether the subject is active in the experimental setting, and 3) whether he actually takes
advantage of the reinforcement when it is presented to him. This differed from his previous experiment in that he used here different levels of shock instead of a single level to investigate the effects of punishment. Three shock levels were used: 30, 80, and 112 volts AC. Murphey found punishment facilitated spatial discrimination performance but he found that only 25% of the performance variance was due to treatment variance and thus concluded that there are an undetermined number of unknown and uncontrolled variables that affect Drosophila discrimination performance. He also found that the least active flies performed best, using time of walking spent between trials, and did not find any direct evidence regarding the incentive value of negative geotaxis.

Finally, Ram (1971) found no evidence of learning when he used shock contingent on the position of the fly's abdomen.

It is curious that as yet there have been no "learning" studies in Drosophila that can stand up to replication. Murphey (1967) has stated, "the nervous system of the Drosophila is rather complex; few would be particularly surprised on anatomical grounds if the animal were to be shown to be capable of learning, especially in the light of the evidence that some other insects which are presumably more primitive than the fruit flies, are able to acquire an instrumental habit." He also points out that both roundworms and flat worms have been shown capable of learning, both of which are supposedly less complex than the fruit fly, although
some controversy still exists.

Ernhart & Sherrick (1959) succeeded in establishing a maze habit in planaria using a T-maze; Best & Rubinstein (1962) obtained the same results using various types of maze. Schmidt (1955) and Krwanek (1956) both succeeded in getting earthworms to learn a maze habit. It has also been shown that the cockroach is capable of learning. Pritchatt & Derrick (1968) found that shock avoidance accomplished through leg lifting could be obtained with relative ease. In this design leg lifting prevented contact with a saline solution that caused the organism's body to complete an electrical circuit. This result was also obtained by Disterhoft (1972) with a similar procedure. The honey bee, presumably with a more primitive nervous system (Murphey, 1967), has been conditioned classically to feed at a dish in response to a neutral stimulus (Wenner & Johnson, 1966), and has the ability to be trained to make a correct response to a color discrimination problem in order to receive food (Bermant & Gary, 1966).

Murphey (1967) offers the following explanations for the inability of the fruit fly to learn: 1) the evolution of learning skipped over the fruit fly but was installed in animals both below it, as shown before, and above it in the phylogenetic order, 2) the fruit fly's evolutionary history has been subject to pressures against learning, thus it would have to be that all the animal's needs were taken care of by the environment without his having to learn, 3) the most
reasonable explanation, the fact that no one has yet designed a task that is most appropriate to their normal behavior.

The purpose of the following two studies was to use a new technique to examine if simple learning was possible in *Drosophila melanogaster*. Electricity was used, but the flies were not shocked as in Murphey's studies. The flies were lured between two horizontal cooper plates one and one half centimeters apart, which made up the top and bottom of one half a rectangular plexiglas box. The other half had a cardboard top and bottom with all areas covered with fiberglass screening. Each copper plate was then connected to a different lead of a high voltage transformer. When the current was turned on while the fly was between the two plates, the fly was oscillated between the two plates rapidly and did not appear to be receiving a shock. A pilot study was run and indicated that this was aversive to the fly and caused no observable physical damage. Such a procedure allowed the flies to be tested in a larger, more natural apparatus where they could walk freely rather than being restricted in a small tube as in other studies of this type.

In the first two experiments, a small light was used as the discriminative stimulus which the fly must learn to avoid. In the second experiment, the gradient of a scent was the discriminative stimulus which the fly must avoid. The rationale behind this was developed from Bolles (1970). The concept is developed that certain responses are learned quickly and others more slowly in avoidance learning because
some are species specific defense reactions which encompass fleeing, freezing, and fighting. Responses closely linked to these three are learned faster because they are basic to the animal's survival in its natural habitat. In the present case, to increase the chances of demonstrating learning, stimuli highly relevant to flies were selected. Both light and scent can serve to attract Drosophila. Drosophila have two sets of eyes, three simple eyes, which are arranged triangularly on the crown of the head and two compound eyes which occupy the sides of the head. The function of the former seems to be as stimulatory organs which accelerate the phototaxic reaction of the flies by increasing the sensitivity of the brain to light-stimuli received through the compound eyes. The compound eyes contain groups of highly specialized photoreceptors which are responsible for the phototaxic reaction in the fly (Wiggleworth, 1966). The odor detection apparatus of the fly is on the antennae in the form of sensory pegs or cones which are sensitive enough for the fly to turn into a column of air with a scent in it at 40 centimeters and walk to the source (Roeder, 1953). The following two studies attempted to demonstrate that Drosophila can learn and also investigated which system, vision or olfaction, serves best in this capacity.
II. METHOD

Experiment I

Subjects:

The subjects were 80 fruit flies (Drosophila melanogaster), descendants of Napa Valley wild type which have been raised Carolina Instant Drosophila Medium no. 67-5002, Carolina Biological Supply Co., in urine bottles. Sex was not controlled but was recorded before such subject was run.

Apparatus:

The apparatus consisted of a rectangular box with the sides constructed of one half cm thick plexiglas. The inside dimensions were four cm long, one cm wide and one and one half cm high. The top and floor of one half of the box was two mm thick cardboard painted black. The other half of the box had a top and floor made of two mm thick copper circuit board. This side delivered the punishment. The cardboard side had fiberglass window screening attached to all sides and back to act as a tactile stimulus as opposed to the smooth sides of the punishment area. Power source was a 10,000 volt AC transformer supplying a maximum .5 ma. A knife switch was used with an on position and an off position which shorted the two copper plates together.
This is a necessary function when using this form of power supply since otherwise the plates would retain a charge even after the power was turned off.

A six mm hole was drilled through the top copper plate through which subjects were introduced into the apparatus. A 12 watt bulb was placed four cm from the end of the punishment side to serve as a discriminative stimulus. The room where the apparatus was located was illuminated only by this bulb.

**Procedure:**

Each subject was taken from its home environment by having it fly out of a small tube inserted in a stopper. It was then trapped in another small plexiglas tube. The sex was then distinguished by the following criteria: 1) the male genitalia are surrounded by heavy dark bristles which do not occur on the female, 2) the presence of sex combs on the front legs of the male and not the female, 3) the tip of the abdomen is rounded on the female whereas on the male it is pointed (Flagg & Noah, 1970).

The subject was then introduced into the apparatus by the hole on the punishment side. The hole was plugged and the fly was given one minute to explore the apparatus before the trial started. If the fly was on the punishment side of the apparatus, the current was turned on for three seconds, or until the fly was thrown clear to the safe side. If the fly was on the safe side, the current was not turned
on until the fly had crossed over to the punishment side. When the fly did cross over, the current was turned on for three seconds or until it was thrown clear again. For the shock to be turned on, the fly had to cross the punishment area with its entire body. If the fly did not leave the punishment area within 15 seconds of the last punishment, the current was turned on again. Each test period lasted for 25 minutes, then the fly was released. The number of shocks received during each minute was recorded. The subject was required to receive at least eight shocks in the first 10 minutes or it was discarded from the experiment for lack of activity.

To be sure that any observed behavior change was due to the punishment and not an attraction to the screening or the black paint a control group was run. The procedure was exactly the same as the experimental group but the shock was disconnected. The data was collected in the same manner as the experimental group. When the fly crossed into the punishment area, a shock received was recorded for that fly but it was not shocked. If it stayed in the punishment area for more than 18 seconds, another shock received was recorded. Thus the data collection for each group was identical.

Method of data analysis:

An adequate measure of avoidance learning would reflect the tendency of the subject to stay away from the area that he is punished in. If avoidance learning was
occurring, the number of encroachments into this area should decrease as more punishment is received. Thus, the total number of shocks received by the subject served as the dependent variable in the first experiment.

Experiment II

Apparatus:

The apparatus was modified by replacing the plexiglas ends of the apparatus with thin cloth screens which allowed air to pass through them. A small animal resusitator was used to control the air flow at a volume of 5 cc at 150 strokes per minute through the apparatus. The room was evenly illuminated from above.

Procedure:

The procedure was the same as in the first experiment with the exception that the fly was placed in the apparatus and after one minute the air flow was turned on and the trial began.

A scent was used to serve the same purpose as the light did in the first experiment, to attract the fly to the punishment side. The air was scented by putting a small amount of fermented banana inside a small tube (Demerec, 1950). The tube was then attached to the output of the resusitator and placed one centimeter from the opening on the punishment end of the apparatus so the air flowed through the apparatus. The data was collected in the same
manner and the dependent variable was the same.

To be sure that any observed behavior change was
due to the punishment and not any attraction in the apparatus
itself, a control group was run as in the first experiment.
The control group was handled as the experimental group,
and all data was collected in the same manner except that
the control group was not shocked.
III. RESULTS

Experiment I

The data was analyzed by breaking up the 25 minute test period into five 5 minute segments for convenience in analysis. A SPF-2.5 analysis of variance (Kirk, 1968) was used. The dependent variable was number of shocks received by the subject in each of the 5 minute segments. It was found that variable A (shock or no shock) was significant ($F=259.28$, df=1/38, $p < .01$), variable B (5 minute segments of time in the apparatus) was significant ($F=82.25$, df=4/152, $p < .01$), and the AB interaction was significant ($F=36.42$, df=4/152, $p < .01$).

The tests of simple main effects were run and are summarized below:

- $A$ at $b_1^*$ = $F= 19.95$, df=1/190, $p < .01$
- $A$ at $b_2$ = $F=185.55$, df=1/190, $p < .01$
- $A$ at $b_3$ = $F=230.41$, df=1/190, $p < .01$
- $A$ at $b_4$ = $F=202.45$, df=1/190, $p < .01$
- $A$ at $b_5$ = $F=190.09$, df=1/190, $p < .01$
- $B$ at $a_1^*$ = $F=112.97$, df=4/152, $p < .01$
- $B$ at $a_2$ = $F= 5.70$, df=4/152, $p < .01$
- $AB$ = $F= 36.42$, df=4/152, $p < .01$

*b1 is first 5 minutes in apparatus, b2 is second 5 minutes in apparatus, etc. a1 is the shock group, a2 is the non-shock group.
The average number of shocks received per 5 minute segments for both control and experimental groups is shown in Figure 1.
Experiment I

Fig. 1. Performance of control vs experimental group over time.
**Experiment II**

The data was analyzed as in Experiment I. It was found that variable A (shock or no shock) was significant ($F=257.28$, $df=1/38$, $p<.01$), variable B (5 minute segments of time in apparatus) was significant ($F=16.25$, $df=4/152$, $p<.01$), and the AB interaction was significant ($F=11.58$, $df=4/152$, $p<.01$).

The tests of simple main effects were run and are summarized below:

- $A$ at $b_1 = F=100.16$, $df=1/190$, $p<.01$
- $A$ at $b_2 = F=147.56$, $df=1/190$, $p<.01$
- $A$ at $b_3 = F=183.87$, $df=1/190$, $p<.01$
- $A$ at $b_4 = F=158.70$, $df=1/190$, $p<.01$
- $A$ at $b_5 = F=159.56$, $df=1/190$, $p<.01$
- $B$ at $a_1 = F=21.46$, $df=4/152$, $p<.01$
- $B$ at $a_2 = F=6.36$, $df=4/152$, $p<.01$
- $AB = F=11.58$, $df=4/152$, $p<.01$

The average number of shocks received per 5 minute segments for both control and experimental groups is shown in Figure 2.
Experiment II

Fig. 2. Performance of control vs experimental group over time.
IV. DISCUSSION

In examining the graphs, it can be seen that in Experiment I the number of shocks received by the experimental group dropped rapidly after the initial five minute segment and asymptotic performance occurred at about 15 minutes. The control group, after a drop in the number of shocks received after the initial five minute segment, kept a fairly stable level of performance for the rest of the experiment.

In Experiment II, the number of shocks received by the experimental group again dropped rapidly after the initial five minute segment and reached asymptotic performance at about 15 minutes. The number of shocks received by the control group gradually dropped throughout the 25 minutes test period.

The data seem to indicate the existence of avoidance learning in *Drosophila*. This is supported most clearly by the significant interaction in both experiments. The two groups, control and experimental, did change differentially in a way that would indicate that learning took place.

However, several factors in addition to learning that may have produced the change are not evident in the data presentation. The manner in which the behavior change occurred involved a marked reduction of activity correlated
with remaining on the safe side. The flies would, upon entering the apparatus, be very active and walk back and forth across the center line. When punishment was initiated the crossing activity would decrease gradually. By the fifth or sixth minute, it would come to a halt and standing in one spot for long periods of time would occur, often right next to the center line on the safe side. There the flies would stand and repeatedly move their front legs toward and then back from the punishment side without receiving punishment. Thus, their movement about the apparatus and consequently the number of shocks received was lowered. Two reasons for this lowered activity could be that this lowering of activity was an unconditioned response of the flies to the punishment, or that the punishment was causing physical damage to the flies, making it difficult to move.

The only other studies using punishment with Drosophila are Murphey's (1967 and 1969) studies. No mention of a drop in activity level is noted, but this could possibly be accounted for due to differences in the administration of the punishment and also different methods (trials vs 25 minute test period) of the two experiments. It could also be possible that the flies learned that being active caused them to be punished so they ceased much of their activity.

To check on the activity levels of the flies after the 25 minute test period, the length of time it took the
subject to exit the apparatus was recorded for 21 subjects in the second experiment. The control flies all left the apparatus in an average of 25 seconds, while none of the experimental subjects left the apparatus within five minutes. Several factors contributed to this discrepancy. First, as mentioned before, the flies reacted to the punishment by reducing their activity level, and second, the exit hole was on the side of the apparatus that the experimental flies had learned to avoid. Thus, it appears that more work is needed to determine if the observed change in behavior was due to learning or to other factors as suggested above.

The possibility that the flies were following odor trials of previously run flies was investigated by alternating control and experimental flies. Data was collected from 18 control flies in Experiment II using two groups of nine flies each. In one group nine control flies were run in succession. In the other group nine control flies were run with two experimental subjects run between each one. The performance of these two groups, one run in succession, the other in alternation with experimental flies, differed significantly only on the initial five minute segment ($T=3.42$, $df=16$, $p<.05$). If the flies were following odor trails, it appears that the effect lasted only for five minutes or that the flies ignored the trail after five minutes. This problem could be solved by waiting a length of time between each trial or adapting the apparatus so that the punishment side could be alternated for every other subject.
The originally planned comparison between the vision and olfaction systems in a learning situation was rendered invalid as the original power source broke down after the first experiment. The replacement proved more powerful than the original and caused differences due to sensory systems to be confounded with this variable. Thus, no attempt will be made at comparing the two systems.

A weak electrical charge was discovered by accident when the experimenter touched one of the plates while the power was on and the plates shorted. It was noticed that the flies seemed to be able to detect the charge because their walking motion changed when they stepped on the copper plates. It appeared though that it was not aversive to the flies because they freely walked on the plates.

More work will be needed to improve the apparatus, assess the effects of activity level changes, and to evaluate stimuli individually to investigate which is singly or in combination most appropriate. In summary, it appears that this is a possibly promising technique to investigate Drosophila avoidance learning but more work is needed in the aforementioned areas before it can be of great use.
V. SUMMARY

Avoidance learning in the fruit fly (Drosophila melanogaster) was investigated using a small rectangular plexiglas box where the flies were oscillated between the top and bottom of one half the box with an electrical force field. In the first experiment, a light was used as the discriminative stimulus and the experimental flies learned to avoid the light significantly more often than the control flies. In the second experiment, a scent gradient was used as the discriminative stimulus and again the experimental flies learned to avoid the scent significantly more often than the control flies. Although both groups of flies learned, it was found that possible physical damage occurred in the flies due to the punishment. It appears that this is a possibly promising technique to investigate Drosophila avoidance learning with more work needed in the area of fly activity and strength of punishment.
REFERENCES

Bastock, M. A. A gene mutation which changes a behavior pattern. Evolution, 1956, 10, 421-439.


Ernhart, E. N. & Sherrick, C. Retention of a maze habit following regeneration in planeria. Paper read at Midwestern Psychological Association, St. Louis, 1954.


